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The Suitability of the Iliac Crest Biopsy in the Element Analysis of Bone and Marrow

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Summary: Bone samples from the iliac crest were taken from 20 subjects and the content of some trace elements (iron, zinc, selenium, cobalt, strontium, aluminium, scandium, rubidium and fluorine) and of the matrix elements calcium, phosphorus and sodium was determined. The samples were taken in accordance with *Burkhardt*'s method, which is often used in hospital for bone biopsies. The sources of errors occurring during the analysis of trace elements using this clinical procedure and the contamination of the samples by blood and the surrounding tissue are discussed. In-vivo activation analysis is also discussed as an alternative method of element analysis of the skeleton.

Die Eignung der Beckenkammbiopsie zur Elementanalyse in Knochen und Mark

Zusammenfassung: Es wurden die Gehalte einiger Spurenelemente (Eisen, Zink, Selen, Cobalt, Strontium, Aluminium, Scandium, Rubidium und Fluor) und der Matrix-Elemente Calcium, Phosphor und Natrium in Knochenproben aus dem Beckenkamm von 20 Personen bestimmt. Die Proben waren nach einem in der Klinik für Knochenbiopsien häufig verwendeten Verfahren nach *Burkhardt* genommen worden. Die Fehlermöglichkeiten bei der Analyse der Spurenelemente unter Verwendung dieses klinischen Verfahrens und die Kontamination der Proben durch Blut und angrenzende Gewebe werden diskutiert. Es wird als alternative Methode zur Elementbestimmung im Skelett die in-vivo-Aktivierungsanalyse diskutiert.

Introduction

Reference was recently made to the significance of the storage of elements in bone tissue and their mobilisation from the skeleton (1, 2). Three aspects were mentioned:

1) Homeostatic functions of bone tissue in the metabolism of the trace elements.

2) The bone as an internal source of trace elements in the course of pathological (e.g. rickets, dialysis) and physiological (old age, pregnancy) changes in the bone mass or composition.

3) The possible role of trace elements in the synthesis and calcification of bone tissue.

However, no standard method for the analysis of trace elements in bone has yet been developed as is the case with the analysis of blood components or other tissues. We became aware of this need whilst investigating the influence of long term dialysis on trace element metabolism.

A standard method for trace element analysis must consider the homogeneity of the samples, contamination by e.g. tools or chemicals and, especially with biological samples, contamination by adjacent tissue or by blood. *Behne* (3) recently summarized the possible sources of error.

In this study we investigated the applicability of the bone biopsy method according to *Burkhardt* (4–6), commonly used in hospitals, for the trace element analysis of bone.

The bone biopsy taken from the iliac crest to determine a number of histological parameters connected with various diseases (osteoporosis, renal osteopathy) is an obvious choice for the source of material for the additional element analysis. The mass of the biopsy obtained using *Burkhardt*'s method is sufficient for trace element analysis even after half has been taken for histological and cytological investigations. When the cortical substance which is thin in this area has been removed, the

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Tab. 1a. Contents of some matrix elements in bone measured by neutron activation analysis and other analytical methods.

	Bone	Tissue*)	Reference mass**)	Matrix elements (fraction × 100) Ca	P	Na
Neutron activation analysis						
Batra & Bewley (9)	?	?	D	23.8	10.3	–
Goode (11)	?	C/Sp	A	31	–	1.01
Liebscher & Smith (12)	?	?	D	–	–	–
Mc Kown et al. (13)	Skull	C/Sp	D	16.6	–	0.69
Söremark & Bergmann (14)	Mandib. Ramus	C	D	25.0 ± 2.4	–	1.41 ± 0.98
This work	Iliac crest	Sp	D, Def	21.3 ± 1.1	9.8 ± 0.6 (21)	0.49 ± 0.09
Other analytical methods (8)			F	10.8–14.8	5	–
			D, Def	17–27.4	17.4	–
			A	35.6–39	15.5	–

*) C = Compacta Sp = Spongiosa **) F = Fresh D = Dry Def = Defatted A = Ashed

sample consists solely of spongy tissue. This tissue, because of the bone remodelling in trabecular bone is more active than in cortical bone, has the advantage of reflecting more rapidly any changes in the composition of bone due to external parameters like medication.

A separation of the cortical from the spongy part is necessary because the trace element content of spongy bone is generally higher than in the compacta. (With fluorine the ratio is 3:1 (20)). A combined analysis of both parts would thus reflect the mass ratio spongiosa/compacta.

Additionally, the element content of bone marrow is of interest, because this tissue represents an important source of contamination for the adjacent bone.

We could find no information about the trace element content of bone marrow in the literature.

The elements investigated were limited by the analytical method used, namely instrumental neutron activation analysis. In addition to the trace elements iron, zinc, selenium, cobalt, strontium, aluminium, scandium, rubidium and fluorine, the contents of the matrix elements, calcium, phosphorus and sodium were determined in bone.

With neutron activation analysis, which is normally too complicated and time-consuming for clinical analysis, no preparation of the bone sample whatsoever (e.g. dissolving or decomposition) is necessary. Since a number of systematic errors in sampling can be excluded when neutron activation analysis is used it has frequently been applied as a reference method.

This analytical method would therefore seem to be suitable for the problem under investigation.

Studies on trace elements in bone have been carried out in the past. Table 1 contains a list of all the studies

Tab. 1b. Contents of some trace elements in bone measured by neutron activation analysis and other analytical methods.

	Bone	Tissue*)	Reference mass**)	Minor and trace elements (mg · kg^{-1}) F	Al
Neutron activation analysis					
Brätter et al. (1)	Iliac crest	Sp	D, Def	658 ± 51	–
Behne et al. (2)	Tibia	C/Sp	D, Def	–	–
Buenfama & Rudelei (10)	?	?	D	–	–
Goode (11)	?	C/Sp	A	–	53–70
Liebscher & Smith (12)	?	?	D	–	–
Mc Kown et al. (13)	Skull	C/Sp	D	–	6541
Söremark & Bergmann (14)	Mandib. Ramus	C	D	–	–
Sowden & Stitch (15)	Iliac crest, rib	C/Sp	A	–	–
van der Mark & Das (16)	?	?	?	800–21600	–
Yamagata (17)	rib	C/Sp	A	–	–
This work	Iliac crest	Sp	D, Def	626 ± 573	19.5 ± 6.1 (21)
Other analytical methods (8)			F	–	5–6540
			D, Def	2800–5600	–
			A	654–28600	30–66

*) C = Compacta Sp = Spongiosa **) F = Fresh D = Dry Def = Defatted A = Ashed

Tab. 2. Composition of the biopsy tools by *Burkhardt* (Manufacturer Straumann, Switzerland). Values in fractions X 100.

	Fe	C	Si	Mn	P	S	Cr	Mo	V	Ni
Hollow milling cutter	79.73	0.92	0.38	0.54	0.023	0.014	17.28	1.02	0.09	–
Pair of tongs for extraction of samples	69.68	0.12	1.0	2.0	–	0.2	18.0	–	–	9.0

known to us on the determination of trace elements in bone tissue using neutron activation analysis (including those investigated in this study). It is accompanied by a survey of the range of values obtained with other analytical methods. The literature data from l.c. (8) were evaluated for this purpose. The bone under investigation, the fraction of compact or spongy tissue and the reference mass were included in the table, as far as they were mentioned in the studies. In view of the fact that the water content varies with bone tissue there would seem to be little point in relating the element masses to the wet weight. In an approximate comparison of the contents related to the dry, fat-free and the ashed bone one can assume a mean ashing loss of 40%.

The wide range of values can certainly not be explained solely by errors in method in the actual analysis, but is also due to the fact that the samples were taken from different parts of the bone (ratio spongiosa/compacta) and to different methods of preparation (e.g. unsatisfactory removal of marrow). This shows how important it is, for the purpose of comparison, to adhere to one method of preparation and to one specific sampling area, which must be defined as clearly as possible.

Materials and Methods

Post mortem samples were taken approximately 1 day after death from 20 persons (11 male, 9 female) in Klinikum Steglitz, Universität Berlin. All had been suffering from diseases which were assumed to have no effect on the bone. The average age was 71 (50–88) years. (We are indebted to Professor Groß of the Dep. of Pathology, Klinikum Steglitz, Freie Universität Berlin, for providing us with the bone samples.) Four samples each were taken from the left and the right iliac crest at intervals of 20 mm. The outermost sample had a distance of 20 mm from the spina iliaca anterior superior. Some of the instruments developed by *Burkhardt* for myelotomy were used.

The samples, of length 20–25 mm, were taken with a hollow milling cutter with an internal diameter of 4 mm (about 300 min^{-1}) and extraction forceps. The composition of the hollow milling cutter and the forceps material, as given by the manufacturers can be found in table 2. Immediately after the samples were taken they were fixed as usual in *Carnoy*'s solution for 4 hours. (0.75 l ethanol, 0.13 l chloroform, 0.13 l glacial acetic acid) and were then stored in polyethylene vials filled with 700 ml/l ethanol. As in the case of the biopsies that we investigated, the cylindrical sample was cut in half down the middle. The blade of the saw used was made of bronze and set with diamonds. The samples were then extracted with ether in a *Soxhlet* apparatus for six hours and subsequently dried at 60 °C for 24 hours. The part of the marrow which was not soluble in ether could easily be removed under the microscope using a fine brush. This part was collected from 3 persons and analysed in the same way.

The samples were first sealed in polyethylene foil and irradiated in the reactor BER II with the help of a fast pneumatic transfer system at a neutron flux of 2×10^{13} cm^{-2} s^{-1} for 20 seconds. This treatment served for the analysis of calcium, phosphorus, aluminium, fluorine and sodium, using element standards. Details of the measurement of F, Ca and Na are published in l.c. (20) and of phosphorus and aluminium in l.c. (21).

After a decay time of only a few days the samples were then sealed in highly pure silica ampoules, activated in the reactor FR 2 in Karlsruhe at a thermal neutron flux density of $5 \cdot 10^{13}$ cm^{-2} s^{-1} for 10 days and after a decay period of approximately 3 months the element contents were assessed according to the relative method using the standards Bovine Liver (NBS 1577), Orchard Leaves (NBS 1571) and Bowens Kale (measuring time 2 hours). The samples remained in the closed silica ampoules while the gamma spectra were being measured.

Minor and trace elements ($mg \cdot kg^{-1}$)

Co	Fe	Rb	Sc	Se	Sr	Zn
0.35 ± 0.1	598 ± 80	–	0.08 ± 0.01	0.47 ± 0.08	162 ± 8	159 ± 7
0.01	8–50	0.02–0.5	0.001	1	–	70–150
–	–	1.9 ± 0.13	–	–	–	–
–	–	–	–	–	–	–
–	–	–	–	–	–	49.9–129
4.6	2041	–	4.6	–	967	–
–	–	5.1	–	–	42 ± 14	117 ± 58
–	–	–	–	–	67–138	–
–	–	–	–	–	–	–
–	–	7.6 ± 2.9	–	–	–	–
0.046 ± 0.037	183 ± 78	< 0.04	0.0014 ± 0.0007	0.13 ± 0.04	79 ± 23	151 ± 22
–	115	–	–	–	55	53–66
0.03–43.5	3–40	–	–	8.9	75–237	50–170
–	707	–	–	–	90–160	187–190

Tab. 3. Arithmetic mean values and standard deviation of the element contents of bone (crista iliaca) of 20 cases.

rsd_1 relative standard deviation of the arithmetic mean value of n_1 samples of one person $\overline{rsd_1}$ arithmetic mean value of rsd_1 from n_2 persons

Section Nr.	Age	Sex	Ca (fraction X 100)				P*) (fraction X 100)	Na (fraction X 100)				F ($mg \cdot kg^{-1}$)				Al ($mg \cdot kg^{-1}$)*)	Co ($\mu g \cdot kg^{-1}$)			
	(a)		mean	$sd._1$	$rsd._1$ (%)	n_1		mean	$sd._1$	$rsd._1$ (%)	n_1	mean	$sd._1$	$rsd._1$ (%)	n_1		mean	$sd._1$	$rsd._1$ (%)	n_1
212	87	♂	22.3	0.6	2.7	5						566	43	8	5		38	34	89	2
214	78	♂	22.9	1.0	4.4	6						1545	657	43	6		13	12	95	3
215	56	♀	20.7	0.7	3.4	6						285	65	23	6		47	14	30	3
218	76	♀	21.3	1.7	8.2	8		0.53	0.10	18	6	834	856	103	8					
219	75	♀	21.2	0.6	2.8	6						453	106	23	6		75	15	21	2
222	83	♀	21.2	0.5	2.4	6						517	79	15	6		16	7	41	3
224	65	♂	20.6	1.2	5.9	8		0.53	0.130	25	7	342	112	33	8					
225	64	♂	20.5	0.7	3.4	5						423	93	22	5		38	42	109	3
226	71	♂	20.6	0.5	2.4	6	10.3					618	88	14	6	29.2	39	5	13	3
232	68	♂	20.9	1.2	5.7	6	10.0					619	155	25	6	28.3	39	19	49	4
234	88	♀	20.3	1.6	7.7	6		0.48	0.096	20	6	451	120	27	6					
239	74	♂	21.6	0.9	4.2	6		0.46	0.0557	12	6	359	73	20	6					
240	50	♂	21.0	0.7	3.3	6	8.87	0.44	0.029	6.5	6	329	60	18	6	14.9	33	20	63	5
249	76	♀	22.1	0.8	3.6	6	9.92	0.51	0.073	14	5	371	90	24	5	12.4	48	11	25	3
263	68	♂	20.9	0.8	3.8	5	9.17	0.51	0.06	12	6	1049	236	23	5	14.8	72	53	74	6
264	68	♀	20.8	1.2	5.8	4	10.3					2794	288	10	4	16.0	86	69	81	6
266	58	♀	21.8	2.1	9.6	6	9.04					332	80	24	6	24.0	27	9	36	3
268	77	♀	22.3	0.7	3.2	6	10.6					677	164	24	6	14.4	33	20	61	4
270	64	♂	22.4	0.7	3.1	6	9.98					346	92	27	6	22.9	41	19	47	4
272	66	♂	21.2	0.6	2.8	6	9.91					328	52	16	6	17.9	27	18	6	4
$\overline{rsd_1}$ (%)/n_2					4.4	20				15	7			26	20				52	16

*) From l.c. (21)

Tab. 3. Arithmetic mean values and standard deviation of the element contents of bone (crista iliaca) of 20 cases.

rsd_1 relative standard deviation of the arithmetic mean value of n_1 samples of one person $\overline{rsd_1}$ arithmetic mean value of rsd_1 from n_2 persons

Section Nr.	Age (a)	Sex	Fe ($mg \cdot kg^{-1}$) mean	sd_1	rsd_1 (%)	n_1	Sc ($\mu g \cdot kg^{-1}$) mean	sd_1	rsd_1 (%)	n_1	Se ($mg \cdot kg^{-1}$) mean	sd_1	rsd_1 (%)	n_1	Sr ($mg \cdot kg^{-1}$) mean	sd_1	rsd_1 (%)	n_1	Zn ($mg \cdot kg^{-1}$) mean	sd_1	rsd_1 (%)	n_1
212	87	♂	77	19	25	2	1.9			1	0.13	0	0	2	119	9.8	8	2	154	1	1	2
214	78	♂	121	56	46	3	1.3	0.6	46	3	0.077	0.015	19	3	26	9	10	3	176	8	8	3
215	56	♀	330	31	10	3	1.5	0.03	2	2	0.150	0.031	21	4	49	6.6	13	3	135	17	13	4
218	76	♀																				
219	75	♀	135	44	32	3	0.8			1	0.100	0.049	49	3	83	22	26	2	165	20	12	3
222	83	♀	76	45	59	3	1.1	0.28	25	2	0.095	0.007	7	3	100	26	25	3	137	5	4	3
224	65	♂																				
225	64	♂	142	38	27	3	0.9	0.14	15	2	0.183	0.084	46	3	77	1	1	2	138	15	11	3
226	71	♂	287	36	13	4	1.7	0.41	24	3					60	6	10	3	177	18	10	4
232	68	♂	147	54	37	4	2.0	0.21	10	2	0.153	0.040	26	3	64	7	10	3	158	22	14	4
234	88	♀																				
239	74	♂																				
240	50	♂	158	15	9	6	0.7	0.24	35	4									164	19	12	6
249	76	♀	135	12	9	4													154	6	4	2
263	68	♂	228	48	21	6	1.4			1									151	16	11	3
264	68	♀	147	25	17	6	3.4			1	0.133	0.015	11	3	117	5.5	5	3	153	24	16	5
266	58	♀	272	43	16	4	2.0	0.7	35	4	0.105	0.021	20	3	81	12	15	3	115	9	8	4
268	77	♀	120	8	7	4	0.9	0.34	36	2	0.140	0.035	25	4	77	10	13	3	136	26	19	4
270	64	♂	252	73	29	4	1.3	1.0	81	4	0.115	0.007	6	3	64	13	21	3	160	9	6	3
272	66	♂	226	56	25	4	1.0	0.24	25	4	0.117	0.025	21	4	62	13	22	3	140	12	9	4
$\overline{rsd_1}$ (%)/n_2					24	16			30	11			23	12			14	13			10	16

*) From l.c. (21)

Results

The arithmetic mean values of the trace element contents in the iliac crest of the 20 persons examined are listed in table 3. This table also includes the standard deviations of the contents of the individual subjects for the discussion of intra-individual distribution.

The phosphorus and aluminium contents have already been published in l.c. (21). In 2 samples with the section numbers 264 and 266 an attempt was made to determine the rubidium contents via a long-term measurement (measuring time: 3 days). In both samples the content was below the detection limit of 40 μg/kg (See "Discussion" on the significance of rubidium as a "scout" element for blood in bone).

Table 4 contains the mean values, the standard deviations, the medians and the percentiles for the samples investigated. By way of comparison, in column 6, the precision of the neutron activation method is given (standard deviation of the arithmetic mean value of 10 identical samples). For the discussion, column 13 shows the relative content of the element in the mineral, taken from l.c. (1).

Table 5 shows the results of the measurements of the trace elements in bone marrow.

Tab. 4. Arithmetic mean values and medians of element contents in bone (crista iliaca) (All samples).

1	2	3	4	5	6	7	8	9	10	11	12	13
Element	Number of samples	Arithmet. mean value	Standard deviation of mean value	Relative standard deviation (%)	Precision*) of NAA-method (%)	Median	Percentiles 2.5%	10%	90%	97.5%	Unit	Relative element content in the mineral (fraction X 100) From l.c. (1)
Ca	118	21.3	1.1	5	5	21.3	19.1	19.9	22.6	23.8	fraction X 100	≈ 100
P	10	9.8	0.6	6	5	9.9		9.04	10.3		fraction X 100	
Na	42	0.49	0.09	17	5	0.49	0.37	0.42	0.61	0.67	fraction X 100	≈ 100
F	118	626	573	92	10	452	232	284	911	2669	$mg \cdot kg^{-1}$	≈ 100
Al	10	19.5	6.1	31	15	16		14.4	28.3		$mg \cdot kg^{-1}$	
Co	58	46	37	81	5	36	6	17	74	174	$\mu g \cdot kg^{-1}$	56
Fe	63	183	78	43	8	165	63.8	90.7	306	325	$mg \cdot kg^{-1}$	70
Rb	2	n.d.(<40)									$\mu g \cdot kg^{-1}$	
Sc	36	1.4	0.7	51	15	1.2	0.5	0.7	2.2	3.0	$\mu g \cdot kg^{-1}$	43
Se	29	0.13	0.04	33	5	0.12	0.07	0.09	0.16	0.19	$mg \cdot kg^{-1}$	33
Sr	36	79	23	29	10	73	46	55	114	126	$mg \cdot kg^{-1}$	≈ 100
Zn	56	151	22	15	5	152	111	123	181	190	$mg \cdot kg^{-1}$	98

*) Standard deviation of the arithmetic mean value of 10 identical samples. NNA = Neutron activation analysis

Tab. 5. Measured element content (arithmetic mean of 3 cases ± standard deviation) of bone marrow (defatted, dry) of the iliac crest.

Ca (fraction X 100)	P (fraction X 100)	Na (fraction X 100)	F ($mg \cdot kg^{-1}$)	Co ($\mu g \cdot kg^{-1}$)	Fe ($mg \cdot kg^{-1}$)	Rb ($mg \cdot kg^{-1}$)	Se ($mg \cdot kg^{-1}$)	Sr ($mg \cdot kg^{-1}$)	Zn ($mg \cdot kg^{-1}$)
8.7 ± 0.5	5.4 ± 0.3	0.05 ± 0.03	243 ± 106	139 ± 12	2080 ± 700	n.d.	0.50 ± 0.06	180 ± 30	280 ± 20

Discussion

It was not the aim of this study to investigate the suitability of a particular analytical method for the detection of matrix and trace elements in bone. The accuracy of the method described above has been tested by an intercomparison of the IAEA (23) and proved to be satisfactory.

In table 4, columns 5 and 6, the relative standard deviation of the measurement values and the precision of the neutron activation analysis method in the determination of the elements are listed side by side. The method is, therefore, sufficiently precise to obtain information on other components contributing to the overall error.

Intra-individual distribution of trace element contents

Mean values and standard deviations were calculated for the individual subjects (tab. 3) after it had been shown that there was no significant pattern in the spatial distribution of the elements across the iliac crest. A measure for intra-individual distribution is the arithmetic mean value $\overline{rsd}$ (tab. 3) of the individual standard deviations rsd. These mean deviations range between 4.4% (calcium) and 52% (cobalt).

The range of values within one individual naturally mirrors all influences on the method of determination, such as sample inhomogeneity, contamination and element loss, and the variations caused by the analytical process itself.

Sample inhomogeneity

Approximately half of the fat-free, dry sample consists of collagen-N and mucopolysaccharide (43% by weight), the remaining 57% being the mineral material (22).

Variations in the contents could be explained if the ratio of the organic to the inorganic part fluctuated, since some of the elements are contained almost exclusively in the mineral part (column 13 in tab. 4).

The mineral part of the investigated samples is, however, very constant. The scatter of the calcium contents can be considered as representative for the scatter of the mineral part; this is only 4.4%. The mean Ca/P ratio measured is 2.17 ($\sigma = 0.17$, $n = 10$) and thus corresponds approximately to that of hydroxyapatite (Ca/P = 2.15), whereas calcium phosphate has a Ca/P ratio of 1.94.

If one assumes hydroxyapatite to be the composition of the mineral phase, the investigated bones contained on average 54% mineral material. It is interesting to note that, with the exception of fluorine, those elements contained to the extent of almost 100% in the mineral (Ca, Na, Sr, Zn), do not show such a wide range of intra-individual distribution, compared with those which are also contained in larger proportions in the organic phase (Co, Fe, Sc, Se). With the exception of iron, these are also, however, the elements with the lowest contents. The special role of iron will be discussed later. Surprisingly, the distribution of fluorine is rather inhomogeneous (mean standard deviation of the F/Ca ratio from 20 persons $\overline{rsd_1} = 25\%$).

External contamination

An important source of contamination, especially with solid samples, is the tools which are used in sampling. Of those elements investigated here the hollow milling cutter, the extraction forceps and the blade of the diamond saw only contain the element iron in larger amounts (hollow milling cutter and forceps, tab. 2). The possibility of contamination with this element cannot, therefore, be excluded. The chromium in the tools, which represents 17–18% (Fe/Cr ratio 5:1), was not found, however, in any of the samples (detection limit for chromium about 1 mg/kg). Contamination with iron is, therefore, smaller than 5 mg/kg with a mean iron content of 183 mg/kg.

Internal contamination

Here the main sources of contamination are blood and bone marrow.

In figure 1 it can be seen that 4 of the elements investigated are present in total blood in a higher concentration than in bone. The content ratio is 11 for iron, 5 for selenium, 2.6 for sodium and greater than 225 for rubidium.

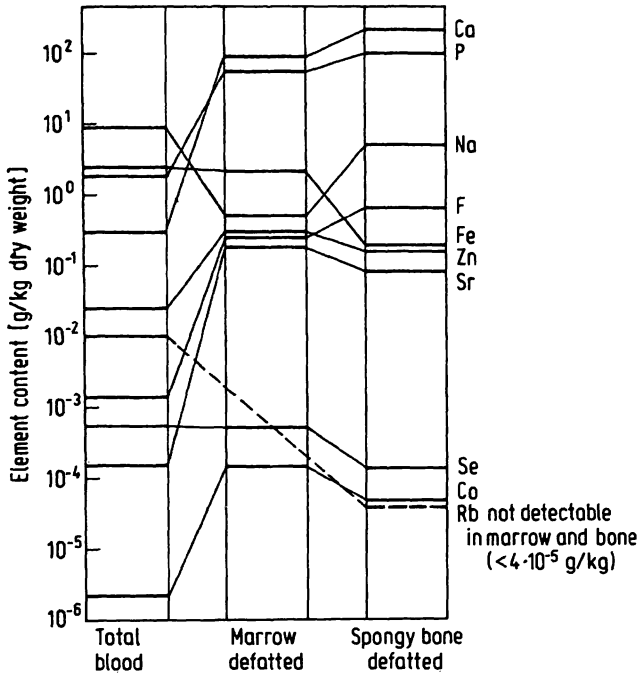

Fig. 1. Comparison of element contents of total blood, marrow and spongy bone. (Values for marrow and spongy bone from the present work: values for total blood from l.c. (8)).

No information is available in the literature on the residual blood content of the bone. In l.c. (7) the blood content of bone in the living organism is given at about 0.05 ml of blood per gramm of bone mass. If the losses due to drying are taken into account this leaves a mass ratio of about 1% of blood in the bone. We suggest, that rubidium is a suitable "scout" element for the determination of the proportion of blood in the bone because of the large content ratio. The limit of detection for rubidium in bone using our method is 40 µg/kg. As the rubidium content in the dry total blood is about 9 mg/kg, the mass ratio blood/bone is less than about 0.4%. With a mean bone iron content of 183 mg/kg this would represent a contamination by iron in the blood corresponding to less than 8.7 mg/kg.

All the trace elements found in the fat-free, dry bone marrow (Al, Rb and Sc were not detected in marrow) were present in larger contents than in the bone (fig. 1). The content ratios are: 11.3 for iron, 1.8 for zinc, 3.8 for selenium, 3.0 for cobalt and 2.3 for strontium. This shows the great importance of the blood marrow as a source of contamination in the analysis of trace elements in bone. As rubidium was not found in the marrow (detection limit 4 mg/kg) it cannot, as with blood, be used as an indicator. It is not possible therefore despite the careful removal of the marrow, to ignore the fact that at least the value given for the iron content in bone might be too high.

Inter-individual distribution

A comparison of the arithmetic mean value (tab. 4, column 3) and the median (column 7) shows a symmetrical distribution with a small standard deviation for the three matrix elements Ca, P and Na. For the element fluorine the well-known unsymmetrical distribution tending towards higher contents was found (arithmetic mean value 626 mg/kg, median 452 mg/kg). Of all the elements investigated fluorine has the widest range of values. The distribution for the contents of the elements Al, Fe, Se, Sc, Sr and Zn is also more or less symmetrical, whereas the irregular distribution of the cobalt contents, on the other hand, is associated with a wide range of values and indicates a hitherto unknown source of contamination.

Comparison with element determination using in-vivo activation analysis

Mainly because of the unknown ratio of trabecular to compact bone a bone biopsy is only to a limited degree representative of the whole skeleton as far as the element content is concerned, even when the choice of the sampling spot is reproducible. Moreover the patient suffers such discomfort as a result of the method of sampling that a biopsy which is taken solely in the interest of determining the element content is certainly not a justifiable diagnostic measure when one considers the present state of the knowledge of trace element metabolism.

On the other hand a number of elements can be determined with the help of total or part body in-vivo neutron activation analysis, the radiation dose which the patient receives being about 1 rem.

A list of applications of this method can be found in l.c. (18, 19). Could in-vivo neutron activation analysis be used to measure the total amount of matrix or trace elements in the skeleton? As far as trace elements are concerned the detection limit of the total body in-vivo method is much too high if the radiation dose is kept at an acceptable level.

The following matrix-elements were determined by total body measurements:

Oxygen, hydrogen, nitrogen, calcium, phosphorus, sodium, potassium and magnesium.

The in-vivo methods do not, however, discriminate between the element content of bone (which is of interest to us here) and that in other tissue and blood. Whereas with an error of only a few percent the calcium mass of the whole body can be equated with that in the bone, this does not apply to phosphorus, and it is not in the least valid for the other elements.

With a biopsy the reference mass – the fat-free, dry bone – can be clearly defined, so that it is possible to obtain information on the element content but not on the mass of the element in the total bone. The in vivo analysis provides the total amount of the calcium in the bone; but it does not reveal the average calcium content of the bone, because the reference mass cannot be determined. In a number of diseases (osteomalacy, renal osteopathy) it is precisely the calcium and phosphorus contents which change.

To date no comparison has been made of the operative risk involved in biopsy sampling with radiation damage after a whole body dose of 1 rem.

Bone Marrow

No measurements of trace element contents in bone marrow have so far been published. The investigation of trace elements in the marrow would appear to be worthwhile, in order to determine the role of trace elements in the important double function of the marrow as haemotopoietic tissue and as the site of osteogenesis.

Marrow itself is a very complex matrix: The proportion of red and yellow marrow is dependent on age and varies from bone to bone. In the iliac crest the proportion of red marrow is about 40% in persons between the age of 40 and 70 years. Whereas about 80–85% of the yellow marrow consists of fat and 15% of water, the red marrow contains in addition to 4% water, and 40% fat about 20% protein (7), as well as erythrocytes, mainly granulocytes and also lymphocytes and other haemic cells.

The contents we measured are related to fat-free, dry marrow and are the mean values of 3 persons (tab. 5). Iron and selenium which have an important function in the haemic cells, are present in the marrow in the same concentration as in the total blood.

Co and Sr are enriched to a very high degree compared with their contents in blood and bone (fig. 1), the content ratio marrow/total blood being 70 for cobalt and 1200 for strontium. These findings could lead to a practical application in the estimation of the radiation burden of marrow through ^{60}Co and ^{90}Sr.

The matrix elements of the bone, Ca, P and also fluorine are in fact present in smaller amounts than in the bone but are extremely enriched compared with the contents in the plasma: (content ratio marrow/plasma in brackets) Ca (87), P (45), F (200).

References

1. Brätter, P., Gawlik, D., Lausch, J. & Rösick, U. (1977) J. Radioanal. Chem. *37*, 393–403.
2. Behne, D., Brätter, P., Keller, C. & Rösick, U. (1974) Radioaktive Isotope in Klinik und Forschung, Bd. 11, 475–480, Urban und Schwarzenberg, München–Berlin.
3. Behne, D. (1981) J. Clin. Chem. Clin. Biochem. *19*, 115–120.
4. Burkhardt, R. (1956) Blut *2*, 267–275.
5. Burkhardt, R. (1966) Klin. Wochenschr. *44*, 326–335.
6. Burkhardt, R. (1966) Blut *14*, 30–36.
7. Report of the Task Group on Reference Man (1975) ICRP – Publication 23, Pergamon Press, Oxford.
8. Iyengar, G. V., Kollmer, W. E. & Bowen, H. J. M. (1978) The Elemental Composition of Human Tissues and Body Fluids, Verlag Chemie, Weinheim, New York.
9. Batra, G. A. & Bewley, D. K. (1973) J. Radioanal. Chem. *16*, 275–280.
10. Buenafama, H. D. & Rudelli, M. D. (1973) J. Radioanal. Chem. *16*, 269–273.
11. Goode, G. C. (1972) Anal. Clin. Acta *58*, 363.
12. Liebscher, K. & Smith, H. (1968) Arch. Environ. Health *17*, 881–890.
13. McKown, D., Kay, M., Gray, D., Abu-Samra, A., Eichor, M. & Vogt, J. (1971) in: Nuclear Methods in Environmental Research (Vogt, J. R., Parkinson, T. F. & Carter, R. L. eds.), University of Missouri, Columbia Mo, pp. 150–157.
14. Söremark, R. & Bergmann, B. (1969) Isotopica *2*, 5–19.
15. Sowden, I. M. & Stitch, S. R. (1957) Biochem. J. *67*, 104–109.
16. van der Mark, W. & Das, H. A. (1972) J. Radioanal. Chem. *13*, 107–124.
17. Yamagata, N. (1962) J. Radioat. Research *3*, 9–30.
18. Cohn, S. H., Cinque, T. J., Dombrowski, C. S. & Letteri, J. M. (1972) J. Lab. Clin. Med. *79*, 978–994.
19. Cohn, S. H. (1978) in Textbook of Nuclear Medicine: Basic Science (Rocha, A. F. G. & Harbert J. C. eds.), Lea & Febiger, Philadelphia pp. 394–406.
20. Behne, D., Brätter, P., Gawlik, D., Keller, C., Möller, J. & Rösick, U. (1977) in: Medizinische Physik Bd. 2 (Lorenz, W. J. ed.), Hüthig Verlag, Heidelberg, pp. 239–252.
21. Gatschke, W. & Gawlik, D. (1980) J. Clin. Chem. Clin. Biochem. *18*, 403–406.
22. Documenta Geigy, Wissenschaftliche Tabellen (1975) Georg Thieme Verlag, Stuttgart.
23. Intercomparison of Minor and Trace Elements in IAEA Animal Bone (H-5). Progress Report No. 1, 1982.

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